10/069772

JC19 Rec'd PCT/PTO 28 FEB 2002

TRANSMITTAL LETTER TO THE UNITED STATES

DESIGNATED/ELECTED OFFICE (DO/E.O./US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER 0050/50669

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP 00/08222	August 23, 2000	September 1, 1999

TITLE OF INVENTION: FATTY ACID DESATURASE GENE FROM PLANTS

APPLICANT(S) FOR DO/E.O./US Ivo FEUSSNER; Ellen HORNUNG; Kathrin FRITSCHE; Nicola PEITZSCH and Andreas RENZ

Applicant herewith submits to the United States Designated/Elected Office (DO/E.O./US) the following items and other information:

1. /X/ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. / / This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. /X/ This express request to begin national examination procedures (35 U.S.C.371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. / / A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. /X/ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).

   a./X/ is transmitted herewith (required only if not transmitted by the International Bureau).
   b./ / has been transmitted by the International Bureau.
   c./ / is not required, as the application was filed in the United States Receiving Office (RO/US0).

6. /X/ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. /X/ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).

   a./X / are transmitted herewith (required only if not transmitted by the International Bureau).
   b./ / have been transmitted by the International Bureau.
   c./ / have not been made; however, the time limit for making such amendments has NOT expired.
   d./ / have not been made and will not be made.

8. /X/ A translation of the amendments to the claims under PCT Article 19(35 U.S.C. 371(c)(3)).

9. /X/ An oath or declaration of the inventor(s)(35 U.S.C. 171(c)(4)).

10./ / A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11./ / An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12./X/ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13./X/ A FIRST preliminary amendment.
   / / A SECOND or SUBSEQUENT preliminary amendment.

14./ / A substitute specification.

15./ / A change of power of attorney and/or address letter.

16./X/ Other items or information.
   International Search Report
   International Preliminary Examination Report

JC19 Rec'd PCT/PTO 28 FEB 2002

10/069772

U.S. Appln. No. (If Known)	INTERNATIONAL APPLN. NO. PCT/EP 00/08222	ATTORNEY'S DOCKET NO. 0050/50669

17. /X/ The following fees are submitted	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO.......................................$890.00	890.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482)......................................$710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).........................$740.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied pro -visions of PCT Article 33(2)-(4).....................$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT = $	890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than / / 20 / /30 months from the earliest claimed priority date (37 CFR 1.492(e)).		

Claims	Number Filed	Number Extra	Rate		
Total Claims	-20		X$18.		
Indep.Claims	-3		X$84.		
Multiple dependent claim(s)(if applicable)			+280.		
TOTAL OF ABOVE CALCULATION			=		
Reduction of ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					
SUBTOTAL			=	890.00	
Processing fee of $130. for furnishing the English translation later than / /20 / /30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					
TOTAL NATIONAL FEE			=	890.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) $40.00 per property =				40.00	
TOTAL FEES ENCLOSED			= $	930.00	

Amount to be refunded: $______

Charged $______

a./X/ A check in the amount of $930.00 to cover the above fees is enclosed.

b./ / Please charge my Deposit Account No.______________in the amount of $______________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c./X/ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-0345. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
KEIL & WEINKAUF
1101 Connecticut Ave., N.W.
Washington, D. C. 20036

____
SIGNATURE

Herbert B. Keil
NAME

18,967
Registration No.

#4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
FEUSSNER et al.) **BOX PCT**
Serial No. 10/069,772)
Filed: February 28, 2002)
For: FATTY ACID DESATURASE GENE FROM PLANTS

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D C. 20231, on:

May 9, 2002
Date of Deposit

Herbert B. Keil
Person Making Deposit

Herbert B Keil
Signature

May 9, 2002
Date of Signature

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT
AND
RESPONSE TO NOTICE TO COMPLY WITH SEQUENCE REQUIREMENTS

Sir:

In response to the Notification to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, a copy of the Sequence Listing in computer readable form is attached hereto. The content of the paper copy of the Sequence Listing and the copy of the Sequence Listing in computer readable form is the same, and includes no new matter.

AMENDMENTS

IN THE SPECIFICATION:

Delete the sequence listing in the specification and substitute with the attached replacement sequence listing as separate pages 1-4.

REMARKS

It is believed that by submitting the present amendment and sequence listing diskette, the application now fully complies with the requirements of 37 CFR 1.821-1.825. Favorable action by the examiner is solicited.

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such account.

Respectfully submitted,

KEIL & WEINKAUF

HBKeil

Herbert B. Keil
Reg. No. 18,967

1350 Connecticut Ave., N.W.
Washington, D.C. 20036
(202)659-0100
HBK/DSK/kas

10/069772

JC19 Rec'd PCT/PTO 28 FEB 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
FEUSSNER et al.) **BOX PCT**
)
PCT/EP 00/08222)
Intl. Filing Date: August 23, 2000)
)
US Serial No.: TO BE ASSIGNED)
)
For: FATTY ACID DESATURASE GENE FROM PLANTS

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination of the above-identified U.S. National Stage application, kindly amend the application as follows.

CLEAN VERSION OF ALL CLAIMS

1. An isolated nucleic acid sequence which encodes a polypeptide with desaturase activity, selected from the following group:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,

b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from the nucleic acid sequence shown in SEQ ID NO: 1,

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and which have at least 75% homology at amino acid level without substantially reducing the enzymatic activity of the polypeptides.

2. An amino acid sequence encoded by a nucleic acid sequence as claimed in claim 1.

3. An amino acid sequence as claimed in claim 2, encoded by the sequence shown in SEQ ID NO: 1.

4. A nucleic acid construct comprising a nucleic acid sequence as claimed in claim 1, where the nucleic acid sequence is linked to one or more regulatory signals.

5. (amended) A vector comprising a nucleic acid sequence as claimed in claim 1 or a nucleic acid construct comprising said

nucleic acid sequence linked to one or more regulatory signals.

6. (amended) An organism comprising at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals.

7. An organism as claimed in claim 6, which is a plant, a microorganism or an animal.

8. (amended) A transgenic plant comprising a functional or nonfunctional nucleic acid sequence as claimed in claim 1 or a functional or nonfunctional nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals.

9. (amended) A process for the preparation of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

10. (amended) A process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into

an oil-producing organism, growing this organism and isolating the oil contained in the organism.

11. (amended) A process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

12. (amended) A process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

13. (amended) A process as claimed in claim 9, wherein the unsaturated fatty acids have an increased calendulic acid content.

14. (amended) A method as claimed in claim 9, wherein the organism is a plant or a microorganism.

15. An unsaturated fatty acid prepared by a process as claimed in claim 9.

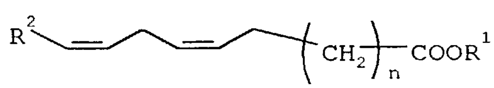
16. A triglyceride with an increased content of unsaturated fatty acids prepared by a process as claimed in claim 10.

17. A saturated fatty acid prepared by a process as claimed in claim 11.

18. A triglyceride with an increased content of saturated fatty acids prepared by a process as claimed in claim 12.

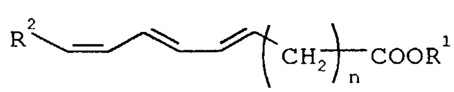
20. (amended) A method for isolating a genomic sequence comprising homology screening with the nucleic acid sequence as claimed in claim 1 or a fragment thereof.

22. An enzyme which converts a fatty acid of the structure I,



(I)

which has two double bonds separated from each other by a methylene group, to give a triunsaturated fatty acid of the structure II,

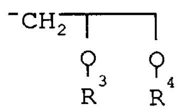


(II),

the three double bonds of the fatty acid being conjugated and the substituents and variables in the compounds of the structures I and

II having the following meanings:

R^1 = hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C_1–C_{10}-alkyl-,



R^2 = substituted or unsubstituted, unsaturated or saturated C_1–C_9-Alkyl-

R^3 and R^4 independently of one another are hydrogen, substituted or unsubstituted, saturated or unsaturated, branched or unbranched C_1–C_{22}-alkylcarbonyl or phospho-,

n = 1 to 14.

MARKED-UP VERSION OF AMENDED CLAIMS

Cancel claims 19 and 21.

5. (amended) A vector comprising a nucleic acid sequence as claimed in claim 1 or a nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals.

6. (amended) An organism comprising at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals.

8. (amended) A transgenic plant comprising a functional or nonfunctional nucleic acid sequence as claimed in claim 1 or a functional or nonfunctional nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals.

9. (amended) A process for the preparation of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

10. (amended) A process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

11. (amended) A process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

12. (amended) A process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct [as claimed in claim 4] comprising said nucleic acid sequence linked to one or more regulatory signals into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

13. (amended) A process as claimed in claim 9 [or 10], wherein the unsaturated fatty acids have an increased calendulic acid content.

14. (amended) A method as claimed in [any of claims 9 to 12] claim 9, wherein the organism is a plant or a microorganism.

20. (amended) [The use of a nucleic acid sequence as claimed in claim 1 or of a fragment thereof] A method for isolating a genomic sequence [via] comprising homology screening with the nucleic acid sequence as claimed in claim 1 or a fragment thereof.

REMARKS

The claims have been amended to eliminate multiple dependency and to place them in better form for U.S. practice. Favorable action on the application is solicited.

Respectfully submitted,

KEIL & WEINKAUF

Herbert B. Keil
Reg. No. 18,967

1101 Connecticut Avenue, N.W.
Washington, D.C. 20036
(202) 659-0100

HBK/kas

0050/50669 41PRTS 10/069772
JC19 Rec'd PCT/PTO 28 FEB 2002

Fatty acid desaturase gene from plants

The present invention relates to a process for the preparation of unsaturated or saturated fatty acids and a process for the preparation of triglycerides with an increased content of unsaturated or saturated fatty acids.

Moreover, the invention relates to a nucleic acid sequence; a nucleic acid construct, a vector and organisms comprising at least one nucleic acid sequence or one nucleic acid construct. Furthermore, the invention relates to saturated or unsaturated fatty acids and triglycerides with an increased content of unsaturated or saturated fatty acids and their use.

Fatty acids and triglycerides have a multiplicity of applications in the food industry, animal nutrition, cosmetics and in the pharmaceutical sector. Depending on whether they are free saturated or unsaturated fatty acids or triglycerides with an increased content of saturated or unsaturated fatty acids, they are suitable for a very wide range of applications; thus, for example, polyunsaturated fatty acids are added to baby formula to increase the nutritional value. The various fatty acids and triglycerides are obtained mainly from microorganisms such as Mortierella or from oil-producing plants such as soya, oilseed rape, sunflowers and others, where they are usually obtained in the form of their triacyl glycerides. Alternatively, they are obtained advantageously from animals, such as fish. The free fatty acids are prepared advantageously by hydrolysis.

Whether oils with unsaturated or with saturated fatty acids are preferred depends on the intended purpose; thus, for example, lipids with unsaturated fatty acids, specifically polyunsaturated fatty acids, are preferred in human nutrition since they have a positive effect on the cholesterol level in the blood and thus on the possibility of heart disease. They are used in a variety of dietetic foodstuffs or medicaments.

Especially valuable and sought-after unsaturated fatty acids are the so-called conjugated unsaturated fatty acids, such as conjugated linoleic acid. A series of positive effects have been found for conjugated fatty acids; thus, the administration of conjugated linoleic acid reduces body fat in humans and animals, and increases the conversion of feed into body weight in the case of animals (WO 94/16690, WO 96/06605, WO 97/46230, WO 97/46118). By administering conjugated linoleic acid, it is also possible to positively affect, for example, allergies (WO 97/32008) or cancer

(Banni et al., Carcinogenesis, Vol. 20, 1999: 1019 - 1024, Thompson et al., Cancer, Res., Vol. 57, 1997: 5067 - 5072).

The chemical preparation of conjugated fatty acids, for example calendulic acid or conjugated linoleic acid, is described in US 3,356,699 and US 4,164,505. Calendulic acid occurs naturally in Calendula officinalis (Ul'chenko et al., Chemistry of Natural Compounds, 34, 1998: 272 - 274). Conjugated linoleic acid is found, for example, in beef (Chin et al., Journal of Food Composition and Analysis, 5, 1992: 185 - 197). Biochemical studies into the synthesis of calendulic acid can be found in Crombie et al., J. Chem. Soc. Chem. Commun., 15, 1984: 953 - 955 and J. Chem. Soc. Perkin Trans., 1, 1985: 2425 - 2434.

Owing to their positive properties, there has been no lack of attempts in the past to make available genes which participate in the fatty acid or triglyceride synthesis for the production, in various organisms, of oils with an altered content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describe a Δ-9-desaturase. WO 93/11245 claims a Δ-15-desaturase, WO 94/11516 a Δ-12-desaturase. Δ-6-Desaturases are described in WO 93/06712 and WO 96/21022. Other desaturases are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144 - 20149, Wada et al., Nature 347, 1990: 200-203 or Huang et al., Lipids 34, 1999: 649 - 659. However, the biochemical characterization of the various desaturases is as yet only insufficient since the enzymes, being the membrane-bound proteins, can only be isolated and characterized with great difficulty (McKeon et al., Methods in Enzymol. 71, 1981: 12141 - 12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777 - 792).

In yeasts, both a shift of the fatty acid spectrum toward unsaturated fatty acids and an increase in productivity were found (see Huang et al., Lipids 34, 1999: 649 - 659, Napier et al., Biochem. J., Vol. 330, 1998: 611 - 614). However, the expression of the various desaturases in transgenic plants did not show the desired success. While it was possible to demonstrate a shift of the fatty acid spectrum toward unsaturated fatty acids, it emerged, simultaneously, that the synthetic productivity of the transgenic plants suffered greatly, viz. lesser amounts of oils were isolated compared with the starting plants.

Thus, there remains a great need for new genes which encode enzymes which participate in the biosynthesis of unsaturated fatty acids and which allow the latter, specifically conjugated

unsaturated fatty acids, to be synthesized and produced on an industrial scale.

It is an object of the present invention to provide other desaturases for the synthesis of unsaturated conjugated fatty acids.

We have found that this object is achieved by an isolated nucleic acid sequence which encodes a polypeptide with desaturase activity, selected from the following group:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,

b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from the nucleic acid sequence shown in SEQ ID NO: 1,

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and which have at least 75% homology at amino acid level without substantially reducing the enzymatic activity of the polypeptides.

A derivative (or derivatives) is/are to be understood as meaning, for example, functional homologs of the enzyme encoded by SEQ ID NO: 1 or its enzymatic activity, viz. enzymes which catalyze the same enzymatic reactions as the enzyme encoded by SEQ ID NO:1. These genes also allow an advantageous preparation of unsaturated conjugated fatty acids. Unsaturated fatty acids are to be understood, in the following text, as meaning mono- and polyunsaturated fatty acids whose double bonds may be conjugated or not conjugated. The sequence given in SEQ ID NO:1 encodes a novel, unknown desaturase which participates in the synthesis of calendulic acid in Calendula officinalis. The enzyme converts (9Z,12Z)octadecadienoic/linoleic acid to (8E,10E,12Z) octadecaconjutrienoic/calendulic acid. This is termed calendulic acid desaturase hereinbelow.

The nucleic acid sequence according to the invention or its fragments can be used advantageously for isolating further genomic sequences by means of homology screening.

The abovementioned derivatives can be isolated, for example, from other eukaryotic organisms such as plants like Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, algae, protozoans such as dinoflagellates, or fungi.

Derivatives or functional derivatives of the sequence given in SEQ ID No.1 are furthermore to be understood as meaning, for example, allelic variants which have at least 75% homology at the derived amino acid level, preferably at least 80% homology, especially preferably at least 85% homology, very especially preferably 90% homology. The homology was calculated over the entire amino acid range. The program used was PileUp (J. Mol. Evolution., 25 (1987), 351-360, Higgins et al., CABIOS, 5 1989: 151 - 153). The amino acid sequence derived from the abovementioned nucleic acid can be seen from the sequence SEQ ID No.2. Allelic variants encompass, in particular, functional variants which can be obtained from the sequence shown in SEQ ID No.1 by means of deletion, insertion or substitution of nucleotides, the enzymatic activity of the derived synthetic proteins being retained.

Such DNA sequences can be isolated from other eukaryotes as mentioned above, starting from the DNA sequence described in SEQ ID No. 1 or parts of these sequences, for example using customary hybridization methods or the PCR technique. These DNA sequences hybridize with the sequences mentioned under standard conditions. It is advantageous to use, for the hybridization, short oligonucleotides, for example from the conserved regions, which can be determined by the skilled worker by comparison with other desaturase genes.

Alternatively, it is possible to use longer fragments of the nucleic acids according to the invention or the full sequences for the hybridization. Depending on which nucleic acid: oligonucleotide, longer fragment or full sequence, or depending on which nucleic acid type, viz. DNA or RNA, is used for the hybridization, these standard conditions vary. Thus, for example, the melt temperatures for DNA:DNA hybrids are approximately 10°C lower than those of equally long DNA:RNA hybrids.

Depending on the nucleic acid, standard conditions are understood as meaning, for example, temperatures between 42 and 58°C in an aqueous buffer solution with a concentration of between 0.1 and 5 x SSC (1 x SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) or additionally in the presence of 50% formamide such as, for example, 42°C in 5 x SSC, 50% formamide. The hybridization conditions for DNA:DNA hybrids are advantageously 0.1 x SSC and temperatures between approximately 20°C and 45°C, preferably

between approximately 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are advantageously 0.1 x SSC and temperatures between approximately 30°C and 55°C, preferably between approximately 45°C and 55°C. These temperatures which are indicated for the hybridization are examples of calculated melting point data for a nucleic acid with a length of approx. 100 nucleotides and a G + C content of 50% in the absence of formamide. The experimental conditions for the DNA hybridization are described in relevant genetics textbooks such as, for example, by Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989 and can be calculated using formulae known to the skilled worker, for example as a function of the length of the nucleic acids, the type of hybrid or the G + C content. The skilled worker can find further information on hybridization in the following textbooks: Ausubel et al. (eds), 1985, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

Derivatives are furthermore to be understood as meaning homologs of the sequence SEQ ID No.1, for example eukaryotic homologs, truncated sequences, simplex DNA of the coding and noncoding DNA sequence or RNA of the coding and noncoding DNA sequence.

Homologs of the sequence SEQ ID No.1 are also to be understood as meaning derivatives such as, for example, promoter variants. These variants can be altered by one or more nucleotide exchanges, by insertion(s) and/or deletion(s), without, however, adversely affecting the functionality or efficacy of the promoters. Moreover, it is possible to increase the efficacy of the promoters by altering their sequence or to exchange them completely by more efficient promoters from other organisms, including other species.

Derivatives are also advantageously to be understood as meaning variants whose nucleotide sequence in the region -1 to -2000 upstream of the start codon was altered in such a way that gene expression and/or protein expression is altered, preferably increased. Moreover, derivatives are also to be understood as meaning variants whose 3' end was altered.

To achieve optimal expression of heterologous genes in organisms, it is advantageous to alter the nucleic acid sequences in accordance with the specific codon usage used in the organism.

The codon usage can be determined readily by using computer evaluations of other, known genes of the organism in question.

The calendulic acid desaturase gene can be combined advantageously in the process according to the invention with other fatty acid biosynthesis genes.

The amino acid sequences according to the invention are to be understood as meaning proteins which contain an amino acid sequence shown in SEQ ID NO: 2 or a sequence obtainable therefrom by the substitution, inversion, insertion or deletion of one or more amino acid residues, the enzymatic activity of the protein shown in SEQ ID NO: 2 being retained or not reduced substantially. The term not reduced substantially is to be understood as meaning all enzymes which still have at least 10%, preferably 20%, especially preferably 30% of the enzymatic activity of the starting enzyme. For example, certain amino acids may be replaced by others with similar physico-chemical properties (spatial dimension, basicity, hydrophobicity and the like). For example, arginine residues are exchanged for lysine residues, valine residues for isoleucine residues or aspartic acid residues for glutamic acid residues. Alternatively, it is possible to exchange the sequence of, add or remove one or more amino acids, or two or more of these measures may be combined with each other.

The nucleic acid construct or nucleic acid fragment according to the invention is to be understood as meaning the sequence given in SEQ ID NO: 1, sequences which are the result of the genetic code and/or their functional or nonfunctional derivatives, all of which have been linked functionally to one or more regulatory signals, advantageously for increasing gene expression. These regulatory sequences are, for example, sequences to which inductors or repressors bind and thus regulate the expression of the nucleic acid. In addition to these novel regulatory sequences, or instead of these sequences, the natural regulation of these sequences upstream of the actual structural genes may still be present and, if desired, may have been genetically altered in such a way that the natural regulation has been switched off and the expression of the genes increased. However, the expression of the gene construct may also have a simpler structure, viz. no additional regulatory signals have been inserted upstream of the sequence or its derivatives and the natural promoter with its regulation has not been removed. Instead, the natural regulatory sequence has been mutated in such a way that regulation no longer takes place and gene expression is increased. These altered promoters may also be placed upstream of the natural gene on their own, in order to increase activity.

In addition, the gene construct can also advantageously contain one or more so-called enhancer sequences functionally linked to the promoter, and these allow an increased expression of the nucleic acid sequence. It is also possible to insert, at the 3' end of the DNA sequences, additional advantageous sequences such as further regulatory elements or terminators. One or more copies of the calendulic acid desaturase gene may be contained in the gene construct.

Advantageous regulatory sequences for the process according to the invention are contained, for example, in promoters such as cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, $lacI^{q}$, T7, T5, T3, gal, trc, ara, SP6, λ-P_R or in the λ-P_L promoter, all of which are advantageously used in Gram-negative bacteria. Other advantageous regulatory sequences are contained, for example, in the Gram-positive promoters amy and SPO2, in the yeast or fungal promoters ADC1, MFα , AC, P-60, CYC1, GAPDH, TEF, rp28, ADH, or in the plant promoters such as CaMV/35S [Franck et al., Cell 21(1980) 285-294], PRP1 [Ward et al., Plant.Mol. Biol.22(1993)], SSU, OCS, lib4, STLS1, B33, nos or in the Ubiquitin promoter. Other advantageous plant promoters are, for example, a benzenesulfonamide-inducible (EP 388186), a tetracyclin-inducible (Gatz et al., (1992) Plant J. 2,397-404), an abscisic-acid-inducible (EP335528) and an ethanol- or cyclohexanone-inducible (WO9321334) promoter. Other plant promoters are, for example, the potato cytosolic FBPase promoter, the potato ST-LSI promoter (Stockhaus et al., EMBO J. 8 (1989) 2445-245), the Glycine max phosphoribosyl pyrophosphate amido transferase promoter (see also gene bank accession number U87999) or a node-specific promoter as described in EP 249676. Advantageous plant promoters are, in particular, those which ensure expression in tissues or parts of the plants in which the biosynthesis of fats or their precursors takes place. Promoters which must be mentioned in particular are those which ensure seed-specific expression such as, for example, the USP promoter, the LEB4 promoter, the phaseolin promoter or the napin promoter.

In principle, all natural promoters with their regulatory sequences as those mentioned above may be used for the process according to the invention. In addition, synthetic promoters may also advantageously be used.

The nucleic acid fragment (= gene construct, nucleic acid construct) may also contain further genes to be introduced into organisms, as this has been described above. These genes can be under separate regulation or under the same regulatory region as the desaturase gene according to the invention. These genes are, for example, other biosynthesis genes, advantageously of the

fatty acid and lipid biosynthesis, which allow increased synthesis. Examples which may be mentioned are the genes for Δ15-, Δ12-, Δ9-, Δ6-, Δ5-desaturase, the various hydroxylases, acetylenase, the acyl-ACP thioesterases, the β-ketoacyl-ACP synthases, the acyltransferases such as diacylglycerol acyltransferase, glycerol-3-phosphate acyltransferase or lysophosphatidic acid acyltransferase or β-ketoacyl-ACP reductases. It is advantageous to use the desaturase genes in the nucleic acid construct, especially the Δ12-desaturase gene.

For expression in a host organism, for example a microorganism such as fungus or a plant, the nucleic acid fragment is advantageously inserted into a vector such as, for example, a plasmid, a phage or other DNA, which vector allows optimal expression of the genes in the host. Examples of suitable plasmids are, in E. coli, pLG338, pACYC184, pBR322, pUC18, pUC19, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III^{113}-B1, λgt11 or pBdCI, in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in Bacillus pUB110, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667, in fungi pALS1, pIL2 or pBB116, in yeasts 2μM, pAG-1, YEp6, YEp13 or pEMBLYe23, or, in plants, pLGV23, pGHlac^{+}, pBIN19, pAK2004, pVKH or pDH51, or derivatives of the abovementioned plasmids. The plasmids mentioned represent a small selection of the plasmids which are possible. Other plasmids are well known to the skilled worker and can be found, for example, in the book Cloning Vectors (Eds. Pouwels P. H. et al. Elsevier, Amsterdam-New York-Oxford, 1985 , ISBN 0 444 904018). Suitable plant vectors are described, inter alia, in "Methods in Plant Molecular Biology and Biotechnology" (CRC Press), Chapter 6/7, pp.71-119.

In addition to plasmids, vectors are also to be understood as meaning all the other vectors which are known to the skilled worker, such as, for example, phages, viruses such as SV40, CMV, baculovirus, adenovirus, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA. These vectors can be replicated autonomously in the host organism or replicated chromosomally. Chromosomal replication is preferred.

The vector advantageously contains at least one copy of the nucleic acid sequence according to the invention and/or of the nucleic acid fragment according to the invention.

To increase the gene copy number, the nucleic acid sequences or homologous genes can be introduced, for example, into a nucleic acid fragment or into a vector which preferably contains the regulatory gene sequences assigned to the genes in question, or

analogously acting promoter activity. Regulatory sequences which are used in particular are those which increase gene expression.

To express the other genes contained, the nucleic acid fragment advantageously additionally contains 3'- and/or 5'-terminal regulatory sequences to increase expression, these sequences being selected for optimal expression, depending on the host organism chosen and the gene or genes.

These regulatory sequences should allow the targeted expression of the genes and protein expression. Depending on the host organism, this may mean, for example, that the gene is expressed and/or overexpressed only after induction, or that it is expressed and/or overexpressed immediately.

The regulatory sequences or factors can preferably have a positive effect on, and thus increase, the gene expression of the genes introduced. Thus, strengthening of the regulatory elements can advantageously take place at the transcriptional level by using strong transcription signals such as promoters and/or enhancers. In addition, however, strengthening of translation is also possible, for example by improving mRNA stability.

In a further embodiment of the vector, the gene construct according to the invention can advantageously also be introduced into the organisms in the form of a linear DNA and integrated into the genome of the host organism by means of heterologous or homologous recombination. This linear DNA may consist of a linearized plasmid or only of the nucleic acid fragment as vector or of the nucleic acid sequence according to the invention.

The nucleic acid sequence according to the invention is advantageously cloned into a nucleic acid construct together with at least one reporter gene, and the nucleic acid construct is introduced into the genome. This reporter gene should allow easy detectability via a growth assay, a fluorescence assay, a chemo assay, a bioluminescence assay or a resistance assay, or via a photometric measurement. Examples of reporter genes which may be mentioned are genes for resistance to antibiotics or herbicides, hydrolase genes, fluorescence protein genes, bioluminescence genes, sugar metabolism genes or nucleotide metabolism genes, or biosynthesis genes such as the Ura3 gene, the Ilv2 gene, the luciferase gene, the β-galactosidase gene, the gfp gene, the 2-deoxyglucose-6-phosphate phosphatase gene, the β-glucuronidase gene, the β-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene or the BASTA (= gluphosinate) resistance gene. These genes allow the

transcriptional activity, and thus gene expression, to be measured and quantified easily. In this way, genome sites which show different productivity can be identified.

In a further advantageous embodiment, the nucleic acid sequence according to the invention may also be introduced into an organism on its own.

If it is intended to introduce, into the organism, other genes in addition to the nucleic acid sequence according to the invention, all can be introduced into the organism in a single vector with a reporter gene, or each individual gene with a reporter gene per vector, it being possible for the various vectors to be introduced simultaneously or in succession.

The host organism advantageously contains at least one copy of the nucleic acid according to the invention and/or of the nucleic acid construct according to the invention.

In principle, the nucleic acid according to the invention, the nucleic acid construct or the vector can be introduced into organisms, for example plants, by all methods known to the skilled worker.

In the case of microorganisms, the skilled worker can find suitable methods in the textbooks by Sambrook, J. et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press, by F.M. Ausubel et al. (1994) Current protocols in molecular biology, John Wiley and Sons, by D.M. Glover et al., DNA Cloning Vol.1, (1995), IRL Press (ISBN 019-963476-9), by Kaiser et al. (1994) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press or by Guthrie et al. Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, 1994, Academic Press.

The transfer of foreign genes into the genome of a plant is termed transformation. The described methods for the transformation and regeneration of plants from plant tissues or plant cells are used for transient or stable transformation. Suitable methods are protoplast transformation by polyethylene-glycol-induced DNA uptake, the use of a gene gun, electroporation, the incubation of dry embryos in DNA-containing solution, microinjection and the agrobacterium-mediated gene transfer. The methods mentioned are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993) 128-143 and by Potrykus, Annu.

Rev. Plant Physiol.Plant Molec.Biol. 42 (1991) 205-225). The construct to be expressed is preferably cloned into a vector which is suitable for transforming Agrobacterium tumefaciens, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711). The transformation of plants with Agrobacterium tumefaciens is described, for example, by Höfgen and Willmitzer in Nucl. Acid Res. 16 (1988) 9877.

Agrobacteria which have been transformed with an expression vector according to the invention can also be used in the known manner to transform plants such as test plants like Arabidopsis or crop plants, in particular oil-containing crop plants such as soya, peanuts, castor, sunflowers, corn, cotton, flax, oilseed rape, coconut palms, oil palms, safflower (Carthamus tinctorius) or cacao, for example by bathing wounded leaves or leaf sections in agrobacterial solution and subsequently culturing them in suitable media.

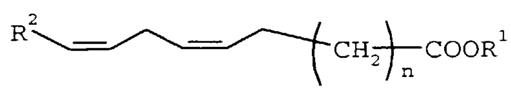
The genetically altered plant cells can be regenerated by all methods known to the skilled worker. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

Suitable organisms or host organisms for the nucleic acid according to the invention, the nucleic acid construct or the vector are, in principle, all organisms which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and which are suitable for the expression of recombinant genes. Examples which may be mentioned are plants such as Arabidopsis, Asteraceae such as Calendula or crop plants such as soya, peanuts, castor, sunflowers, corn, cotton, flax, oilseed rape, coconut palms, oil palms, safflower (Carthamus tinctorius) or cacao, microorganisms such as fungi, for example the genus Mortierella, Saprolegnia or Pythium, bacteria such as the genus Escherichia, yeasts such as the genus Saccharomyces, algae or protozoans such as dinoflagellates such as Crypthecodinium. Preferred organisms are those which are naturally capable of synthesizing oils in substantial amounts, like fungi such as Mortierella alpina, Pythium insidiosum or plants such as soya, oilseed rape, flax, coconut palms, oil palms, safflower, castor, Calendula, peanuts, cacao or sunflowers, or yeasts such as Saccharomyces cerevisiae, with soya, oilseed rape, flax, sunflowers, Calendula or Saccharomyces cerevisiae being especially preferred. In principle, transgenic animals, for example Caenorhabditis elegans, are also suitable as host organisms.

Another embodiment according to the invention are, as described above, transgenic plants which contain a functional or a nonfunctional nucleic acid or a functional or nonfunctional nucleic acid construct. The term nonfunctional is to be understood as meaning that an enzymatically active protein is no longer synthesized since the natural gene has been inactivated. In addition, the term nonfunctional nucleic acids or nucleic acid constructs is also to be understood as meaning a so-called antisense DNA which leads to transgenic plants which show a reduction in, or lack, enzymatic activity. The antisense technology, specifically when combining, in the antisense DNA, the nucleic acid sequence according to the invention with other fatty acid synthesis genes, allows the synthesis of triglycerides with an elevated content of saturated fatty acids, or saturated fatty acids. Transgenic plants are to be understood as meaning single plant cells and their cultures on solid media or in liquid culture, parts of plants and entire plants.

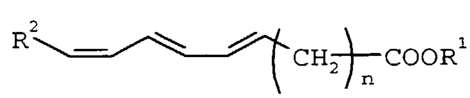
The use of the nucleic acid sequence according to the invention or of the nucleic acid construct according to the invention for the generation of transgenic plants is therefore also subject matter of the invention.

The invention furthermore relates to an enzyme which converts a fatty acid of the structure I,



(I)

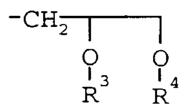
which has two double bonds separated from each other by a methylene group, to give a triunsaturated fatty acid of the structure II,



(II),

the three double bonds of the fatty acid being conjugated and the substituents and variables in the compounds of the structures I and II having the following meanings:

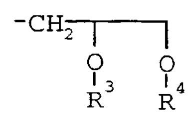
R^1 = hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C_1-C_{10}-alkyl-,



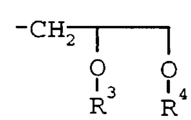
R^2 = substituted or unsubstituted, unsaturated or saturated C_1-C_9-Alkyl-

R^3 and R^4 independently of one another are hydrogen, substituted or unsubstituted, saturated or unsaturated, branched or unbranched C_1-C_{22}-alkylcarbonyl or phospho-,

n = 1 to 14, preferably 1 to 8, especially preferably 4 to 6, very especially preferably 6.

R^1 in the compounds of the formula I and II is hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C_1-C_{10}-alkyl-, or 

Alkyl radicals which may be mentioned are substituted or unsubstituted, branched or unbranched C_1-C_{10}-alkyl chains such as, for example, methyl, ethyl, n-propyl, 1-methylethyl, n-butyl, 1-methylpropyl-, 2-methylpropyl, 1,1-dimethylethyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 1-ethylpropyl, n-hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, n-heptyl, n-octyl, n-nonyl or n-decyl.

Preferred radicals for R^1 are hydrogen and 

R^2 in the compounds of the formula I and II denotes substituted or unsubstituted, unsaturated or saturated C_1-C_9-alkyl-.

Alkyl radicals which may be mentioned are substituted or unsubstituted, branched or unbranched C_1-C_9-alkyl chains such as, for example, methyl, ethyl, n-propyl, 1-methylethyl, n-butyl, 1-methylpropyl-, 2-methylpropyl, 1,1-dimethylethyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl,

1-ethylpropyl, n-hexyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, n-heptyl, n-octyl or n-nonyl. C_1-C_5-alkyl is preferred, C_5-alkyl is especially preferred.

R^3 and R^4 independently of one another are hydrogen, substituted or unsubstituted, saturated or unsaturated, branched or unbranched C_1-C_{22}-alkylcarbonyl- or phospho-.

C_1-C_{22}-alkylcarbonyl such as methylcarbonyl, ethylcarbonyl, n-propylcarbonyl, 1-methylethylcarbonyl, n-butylcarbonyl, 1-methylpropylcarbonyl, 2-methylpropylcarbonyl, 1,1-dimethylethylcarbonyl, n-pentylcarbonyl, 1-methylbutylcarbonyl, 2-methylbutylcarbonyl, 3-methylbutylcarbonyl, 1,1-dimethylpropylcarbonyl, 1,2-dimethylpropylcarbonyl, 2,2-dimethylpropylcarbonyl, 1-ethylpropylcarbonyl, n-hexylcarbonyl, 1-methylpentylcarbonyl, 2-methylpentylcarbonyl, 3-methylpentylcarbonyl, 4-methylpentylcarbonyl, 1,1-dimethylbutylcarbonyl, 1,2-dimethylbutylcarbonyl, 1,3-dimethylbutylcarbonyl, 2,2-dimethylbutylcarbonyl, 2,3-dimethylbutylcarbonyl, 3,3-dimethylbutylcarbonyl, 1-ethylbutylcarbonyl, 2-ethylbutylcarbonyl, 1,1,2-trimethylpropylcarbonyl, 1,2,2-trimethylpropylcarbonyl, 1-ethyl-1-methylpropylcarbonyl and 1-ethyl-2-methylpropylcarbonyl, heptylcarbonyl, nonylcarbonyl, decylcarbonyl, undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl or n-eicosylcarbonyl.

Preferred substituents for R^3 and R^4 are saturated or unsaturated C_{16}-C_{22}-alkylcarbonyl.

Examples of substituents of the abovementioned radicals which may be mentioned are halogen such as fluorine or chlorine, alkyl or hydroxyl.

In the conversion with the enzyme according to the invention, one double bond is introduced into the fatty acid and one double bond is shifted so that the three double bonds which participate in the reaction are conjugated. Furthermore, one double bond is isomerized (from cis to trans).

The enzyme (= calendulic acid desaturase) advantageously catalyzes the conversion of linoleic acid (18:2, 9Z,12Z) to calendulic acid (18:3, 8E,10E,12Z). The enzyme introduces a trans double bond at position C8 and causes the specific shift of a cis double bond in position C9 to a trans double bond in position C10, the isomerization being effected regiospecifically. A possible hypothetical reaction mechanism is shown in Fig. 1. After deprotonation at C8 of the linoleic acid and a rearrangement of the free radical to C10, the elimination of water leads to a deprotonation at C11 and thus to the formation of calendulic acid. Simultaneously, bound FeIV is reduced to FeIII. Fig. 1 shows the hypothetical mechanism for (8,11)-linoleoyl desaturase (calendulic acid desaturase), modified after Svatos, A et al. (Insect Biochemistry and Molecular Biology 29,1999:225-232) based on the proposed catalytic mechanism for Ricinus Δ9 desaturase (Lindqvist, Y et al., EMBO Journal 15, 1996:4081-4092). Suitable substrates are still 6Z,9Z,12Z, 18:3-fatty acid and 9Z,12Z,15Z, 18:3-fatty acid, which, in turn, are then reacted to give 6Z,8E,10E,12Z- and 8E,10E,12Z,15Z-fatty acids, respectively.

The invention furthermore relates to a process for the preparation of unsaturated fatty acids, which comprises introducing at least one above-described nucleic acid sequence according to the invention or at least one nucleic acid construct according to the invention into a preferentially oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

The invention also includes a process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one above-described nucleic acid sequence according to the invention or at least one nucleic acid construct according to the invention into a preferentially oil-producing organism, growing this organism and isolating the oil contained in the organism.

Both processes advantageously allow the synthesis of fatty acids of triglycerides with an increased content of unsaturated fatty acids such as calendulic acid.

The invention furthermore relates to a process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional abovementioned nucleic acid sequence according to the invention or at least one nonfunctional nucleic acid construct according to the invention into an oil-producing organism, growing this organism, isolating the oil contained in

the organism and liberating the fatty acids contained in the oil, and to a process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional abovementioned nucleic acid sequence according to the invention or at least one nonfunctional nucleic acid construct according to the invention into an oil-producing organism, growing this organism and isolating the oil contained in the organism. Both processes involve the use of the so-called antisense technology (see above), or the inactivation of the lateral synthesis genes.

Examples of organisms for the abovementioned processes are plants such as Arabidopsis, soya, peanuts, castor, sunflowers, corn, cotton, flax, oilseed rape, coconut palms, oil palms, safflower (Carthamus tinctorius) or cacao, microorganisms such as the fungi Mortierella, Saprolegnia or Pythium, bacteria such as the genus Escherichia, yeasts such as the genus Saccharomyces, algae or protozoans such as dinoflagellates, for example Crypthecodinium. Preferred organisms are those which can naturally synthesize oils in substantial amounts, such as fungi, for example Mortierella alpina, Pythium insidiosum, or plants such as soya, oilseed rape, flax, coconut palms, oil palms, safflower, castor, Calendula, peanuts, cacao or sunflowers, or yeasts such as Saccharomyces cerevisiae; soya, oilseed rape, flax, sunflowers, Calendula or Saccharomyces cerevisiae are especially preferred.

Depending on the host organism, the organisms used in the processes are grown or cultured in the manner known to those skilled in the art. As a rule, microorganisms are grown in a liquid medium which contains a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, a phosphate source such as potassium hydrogen phosphate, trace elements such as iron salts, manganese salts, magnesium salts and, if required, vitamins, at temperatures between 0°C and 100°C, preferably between 10°C and 60°C, while gassing in oxygen. The pH of the liquid medium can be maintained at a fixed value, i.e. the pH is regulated while culture takes place. However, the microorganisms may also be cultured without pH regulation. Culturing can be effected by the batch method, the semi-batch method or continuously. Nutrients may be supplied at the beginning of the fermentation or fed in semicontinuously or continuously.

Post-transformation, plants are first regenerated as described above and then grown or planted as usual.

After the organisms have been grown, the lipids are obtained in the usual manner. To this end, the organisms can first be harvested and then disrupted, or they can be used directly. It is advantageous to extract the lipids with suitable solvents such as apolar solvents, for example hexane, or polar solvents, for example ethanol, isopropanol, or mixtures such as hexane/isopropanol, phenol/chloroform/isoamyl alcohol, at temperatures between 0°C and 80°C, preferably between 20°C and 50°C. As a rule, the biomass is extracted with an excess of solvent, for example with an excess of solvent to biomass of 1:4. The solvent is subsequently removed, for example by distillation. The extraction may also be carried out with supercritical CO_2. After the extraction, the remainder of the biomass can be removed, for example, by filtration. Standard methods for the extraction of fatty acids from plants and microorganisms are described in Bligh et al. (Can. J. Biochem. Physiol. 37, 1959: 911-917) or Vick et al. (Plant Physiol. 69, 1982: 1103-1108).

The crude oil thus obtained can then be purified further, for example by removing cloudiness by adding polar solvents such as acetone or apolar solvents such as chloroform, followed by filtration or centrifugation. Further purification via columns or other techniques is also possible.

To obtain the free fatty acids from the triglycerides, the latter are hyrolyzed in the customary manner, for example using NaOH or KOH.

The invention furthermore relates to unsaturated or saturated fatty acids and triglycerides with an increased content of saturated or unsaturated fatty acids which have been prepared by the abovementioned processes, and to their use for the preparation of foodstuffs, animal feed, cosmetics or pharmaceuticals. To this end, they are added to the foodstuffs, animal feed, cosmetics or pharmaceuticals in the customary quantities.

The invention is illustrated in greater detail in the examples which follow:

Examples

A cDNA was cloned from Calendula officinalis mRNA using RT-PCR and RACE techniques. When expressing this cDNA in yeast, linoleic acid is converted into the octadecaconjutriene calendulic acid (8E, 10E, 12Z). As far as we know, this is the first time that a calendulic acid desaturase has been described. The enzyme causes

a regiospecific shift of a *cis* double bond in position C9 to a *trans* double bond in position C10 and introduces a new *trans* double bond at position C8.

Transgenic yeasts and plants with an increased expression of calendulic acid desaturase cDNA show calendulic acid in their lipids.

Example 1: Isolation of RNA from Calendula officinalis seeds

In order to be able to isolate cDNA clones for calendulic acid desaturase by means of PCR, RNA was isolated from Calendula officinalis seeds. Owing to the high fat content of the seeds, it was impossible to use standard protocols; the following method was used instead:

Using a pestle and mortar, 20 g of plant material were ground in liquid nitrogen to give a powder. 100 ml of extraction buffer I [100 mM tris/HCl, pH 7.5, 20 mM EDTA, 2% (w/v) lauryl sarcosyl, 4 M guanidinium thiocyanate, 5% (w/v) PVP (= polyvinyl-pyrrolidone), 1% (v/v) β-mercaptoethanol] were added, and the batch was mixed immediately and homogenized. The solution was transferred into 50-ml-vessels and shaken for approximately 15 minutes. After centrifugation for 10-15 minutes at 4,000 g, the fatty layer or fat drops which had risen to the top were removed and the supernatant was transferred into fresh vessels. This was followed by extraction with 1 volume of phenol/chloroform/isoamyl alcohol (= PCI, 25:24:1) and one extraction with chloroform; in each case, the mixture was shaken for 15 minutes and then centrifuged. The upper, aqueous phase was removed, placed on an 8-ml-CsCl cushion (5 M CsCl) and centrifuged for 18 hours at 18°C and 100,000 g. The supernatant was decanted off and the RNA precipitate was dried briefly. After a washing step with 70% ethanol, the RNA was dissolved in a mixture of 7.5 ml extraction buffer II (100 ml tris/HCl, pH 8.8, 100 mM NaCl, 5 mM EDTA, 2% SDS) and 10 ml of PCI, shaken for 15 minutes and centrifuged. The upper, aqueous phase was extracted with chloroform and then an equal volume of 5 M LiCl was added. The RNA was precipitated overnight at 4°C. The mixture was then centrifuged for 60 minutes at 12,000 g and 4°C. The precipitate was washed twice with 70% ethanol, dried and finally taken up in 500 µl of H_2O.

mRNA was isolated from the resulting Calendula total RNA using the Poly-Attract Kit (Promega, Mannheim) following the manufacturer's instructions. 1 µg of this mRNA was translated into cDNA with the SuperscriptII reverse transcriptase by Gibco

BRL (Eggenstein) using 200 pmol of oligo-dT primer following the manufacturer's instructions and employed as template in a polymerase chain reaction (PCR).

Example 2 : Isolation and cloning of the Calendula officinalis calendulic acid desaturase

In order to isolate, from Calendula officinalis, DNA sequences which encode a calendulic acid desaturase, various degenerate oligonucleotide primers were derived from amino acid sequences of the conserved histidine boxes of various Δ12 desaturases.

Primer A: 5' - CCD TAY TTC TCI TGG AAR WWH AGY CAY CG - 3'
forward primer, derived from the amino acid sequence
P Y F S W K Y/I S H R

Primer B: 5' - CCA RTY CCA YTC IGW BGA RTC RTA RTG - 3'
reverse primer, derived from the amino acid sequence
H Y D S S/T E W D/N W

The letters in primers A and B have the following meaning:

R = A/G
Y = C/T
W = A/T
H = A/C/T
B = C/G/T
D = A/G/T
I = inositol

In a PCR with Calendula simplex cDNA (prepared as described in Example 1) as template, a DNA fragment with a length of 470 bp was amplified using primers A and B. The following PCR program was used:

1. 2 min 94 °C
2. 30 sec 94 °C
3. 45 sec 50 °C (annealing temperature)
4. 1 min 72 °C
   10 x 2. to 4.
5. 0 sec 94 °C
6. 45 sec 50 °C
7. 1 min 72 °C, time increment 5 sec per cycle
   20 x 5. to 7.
8. 2 min 72 °C

The TfI DNA polymerase from Biozym (Hess. Oldendorf) was used for the amplification. The 470 bp DNA fragment was cloned into the vector pCR 2.1-TOPO with the aid of the TOPO TA Cloning Kit (Invitrogen, Carlsbad, USA) and sequenced. The sequence of the 470 bp fragment corresponded to the sequence of nucleotide 466 to 893 of SEQ ID NO:1.

Example 3: Obtaining and sequencing complete cDNA clones

In order to obtain a full-length clone, the fragment was extended by means of 5'- and 3'-RACE (rapid amplification of cDNA ends). Starting from 1 μg of mRNA (isolated as described in Example 1), duplex cDNA was prepared using the "Marathon cDNA Amplification Kit" by CLONTECH (Heidelberg). After ligation of the adaptor, 5'- and 3'-RACE was carried out using the following primers:

Specific primers for 5'-RACE:

Primer C 5' - GTG AGG GAG TGA GAG ATG GGT GTG GTG C - 3'
Primer D 5' - AAC ACA CTT ACA CCT AGT ACT GGA ATT G - 3'

Specific primers for 3'-RACE:

Primer E 5' - TAT TCC AAA CTT CTT AAC AAT CCA CCC G - 3'
Primer F 5'- CAA TTC CAG TAC TAG GTG TAA GTG TGT T - 3'

First, a PCR was carried out with the adaptor-ligated duplex cDNA and primer C or E; then, a second PCR was carried out with primer D or F and a 1:50 dilution of the PCR product from the reaction with primer C or E as template.

The RACE-PCR was carried out using the following program:

1. 1 min 94°C
2. 30 sec 94°C
3. 3 min 68°C
   10 x 2. - 3.
4. 30 sec 94°C
5. 30 sec 65°C
6. 3 min 68°C
   25 x 4. - 6.
7. 5 min 68°C

The resulting DNA fragments were cloned into pCR 2.1-TOPO with the aid of the TOPO TA Cloning Kit (Invitrogen, Carlsbad, USA) and sequenced. The 5'-RACE product extended over the start codon

into the 5′-untranslated region (5′-UTR), and the 3′-RACE over the stop codon into the 3′-UTR).

The composite sequence composed of the first PCR product and the RACE product is shown in SEQ ID NO: 1. The encoding region extends from nucleotide 42 (start codon) to 1175 (stop codon). The 5′- and 3′- UTRs were only sequenced as simplexes, so that individual sequencing mistakes are possible here.

In order to obtain an uninterrupted full-length clone, a PCR was carried out using the Expand High Fidelity System (Boehringer, Mannheim) and the primers G and H, with Calendula cDNA (see Example 1) as template.

Primer G 5′ - ATTA**GAGCTC**ATGGGTGCTGGTGGTCGGATGTCG - 3′
forward primer (with **SacI** cleavage site)

Primer H 5′ - ATTA**CTCGAG**TGACATACACCTTTTTGATTACATCTTG - 3′
reverse primer (with **XhoI** cleavage site)

The PCR was carried out using the following program:

1. 2 min 94°C
2. 30 sec 94°C
3. 35 sec 63°C
4. 2 min 72°C
   10 x 2. - 4.
5. 30 sec 94°C
6. 35 sec 63°C
7. 2 min 72°C, time increment 5 seconds per cycle
   15 x 5. - 7
8. 2 min 72 °C.

The 1.2 kb PCR product was cloned into the vector pGEM-T (Promega, Mannheim) and transformed into E. coli DH10B. The insert DNA was sequenced as duplex using a 373 DNA sequencer (Applied Biosystems). To this end, the following sequence-specific primers were used in addition to reverse primer and -21 primer:

Primer I: 5′ - CGG TCT TCT CGC TGT ATT - 3′

Primer J: 5′ - ATT ACC CAA GCT GCC C - 3′

The complete DNA sequence of calendulic acid desaturase (CalDes) is identical to the section from nucleotide 42 to 1193 of SEQ ID NO:1. The sequence encompasses the encoding region and a short section of the 3'-UTR.

A comparison of the derived amino acid sequence of Co-CalDes (SEQ ID NO:2) with annotated protein sequences of the SWISS-PROT and SP-TREMBL databases demonstrated the highest homology to a Crepis alpina Δ12-acetylenase (SP_PL: 081931, 74% identical amino acids), a Crepis palaestina Δ12-epoxygenase (SP_PL: 065771, 73% identical amino acids) and a Borago officinalis Δ12-desaturase (SP_PL: 082729, 62% identical amino acids) over the entire encoding region. The sequence comparisons are shown in Fig. 2. Fig. 2 shows a comparisons of the amino acid sequences of Co-CalDes with Crepis alpina Δ12-acetylenase (Ca-Acetyl), Crepis palaestina Δ12-epoxygenase (Cp-Epoxy) and Borago officinalis Δ12-desaturase (Bo-Des).

Example 4: Expression of calendulic acid desaturase in yeast

In a first approach, the encoding region of the cDNA was cloned in a yeast expression vector and expressed in S. cerevisiae, in order to demonstrate the functionality of CalDes. The calendulic acid desaturase produced in the yeast was meant to convert added linoleic acid into calendulic acid. The latter, in turn, was to be detected by HPLC in hydrolyzed lipid extracts.

In a second approach, the A. thaliana Δ12-desaturase FAD2 (Kajiwara et al., Appl. Environ. Microbiol., 62, 1996: 4309 - 4313) was expressed in yeast in addition to CalDes, so that the yeast cells endogenously produce linoleic acid which, in turn, can be converted into calendulic acid owing to the activity of CalDes. The calendulic acid, in turn, was to be detected by HPLC.

All solid and liquid media for yeast were prepared by protocols of Ausubel et al. (Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1995).

The CalDes cDNA was excised from the vector pGEM-T by restriction digest with SacI/XhoI and cloned into the SacI/XhoI-cut shuttle vector pYES2 (Invitrogen, Carlsbad, USA), and the resulting vector pYES2-CalDes was transformed into E. coli XL1 blue. After another plasmid preparation with the aid of the Plasmid Maxi Kit (QIAGEN), pYES2-CalDes was transformed into S. cerevisiae INCSv1 (Invitrogen, Carlsbad, USA) with the aid of the polyethylene glycol method (Von Pein M., PhD thesis, Heinrich Heine-

Universität Düsseldorf, 1992), where the expression of the CalDes cDNA was under the control of the GAL1 promoter.

In order to be able to express, in the second approach, not only CalDes, but also FAD2, in yeast, the encoding region of the FAD2 gene was first amplified via PCR (protocol see Primers G and H) from A. thaliana cDNA with the aid of Tfl polymerase (Biozym). The following primers were used for this purpose:

Primer K: 5′ - AAA**CTCGAG**ATGGGTGCAGGTGGAAGAATGCCGG - 3′
forward primer (**XhoI** cleavage site)

Primer L: 5′ - AAA**AAGCTT**TCATAACTTATTGTTGTACCAGTACACACC - 3′
reverse primer (**HindIII** cleavage site)

The resulting PCR product was subjected to a restriction digest with XhoI/HindIII and then cloned into the XhoI/HindIII-cut yeast expression vector pESC-Leu (Stratagene), where the FAD2 DNA was under the control of the GAL1 promoter.

The expression of CalDes in S. cerevisiae INCSv1 was carried out using a modification of the procedure of Avery et al. (Appl. Environ. Microbiol., 62, 1996: 3960 - 3966) and Girke et al. (The Plant Journal, 5, 1998: 39 - 48). To prepare a starter culture, 10 ml of YPAD medium were inoculated with a single colony and the culture was incubated for 48 hours at 30°C at 200 rpm. Then, the cell culture was washed in 1 x YPA medium without sugar and centrifuged. The pelleted cells were resuspended in 2 ml of minimal medium without supplements and without sugar. 100 ml of minimal medium (dropout powder, 2% raffinose, 1% Tergitol NP40) in 500-ml-Erlenmeyer flasks were inoculated with 1 ml of this cell suspension and the culture was grown at 30°C and 200 rpm. At an OD_{600} of 0.5, 2% (w/v) of galactose were added and (in the case of the first batch) 0.003% of linoleic acid (3% stock solution in 5% Tergitol NP40). The cells were grown on until the stationary phase had been reached. They were then washed in minimal medium without supplements and stored at -20°C.

Example 5: Lipid extraction and HPLC analysis of the fatty acids from transgenic yeast

The yeast cells were suspended in 30 ml of HIP solution (0.1 mM 2.6-di-tert-butyl-4-methylphenol in hexane: isopropanol (3:2 v/v)), acidified with 150 µl of concentrated HCl and homogenized in an Ultra-Turrax (1 min, 24,000 rpm). The samples were then shaken for 10 minutes at 4°C and centrifuged for 10 minutes at 5,000 g and 4°C. The supernatant was transferred into a fresh

container and made up to 47.5 ml with 0.38 M K_2SO_4. The samples, in turn, were shaken for 10 minutes at 4°C and centrifuged (see above). The hexane phase was withdrawn and evaporated to dryness under a stream of N_2. The residue was dissolved in 20 µl of chloroform. For the alkaline hydrolysis of fatty acid esters, 400 µl of methanol and 80 µl of 40% strength (w/v) KOH solution were added and the sample was incubated for 20 minutes at 60°C under argon. The sample was subsequently cooled to room temperature, acidified to pH 3.0 with 35 µl of concentrated HCl and separated by HPLC.

The free fatty acids were separated using an ET 250/4 Nucleosil 120-5 C18-column (Macherey & Nagel). The mobile phase used was methanol:H_2O:glacial acetic acid (85:15:0.1 v/v/v). The separation was carried out at a flow rate of 1 ml/min and 25°C, and the absorption was measured at 268 nm to detect the conjutrienes.

Fig. 3 shows the elution profiles of the lipid extracts from transformed yeast cells following alkaline hydrolysis (Fig. 3B, elution profile of S. cerevisiae INCSv1 transformed with A. thaliana FAD2 DNA, and C, elution profile of *S. cerevisiae* INCSv1 transformed with Calendula officinalis pYES2-CalDes), and the elution profile of a calendulic acid standard (Fig. 3A). Calendulic acid has a retention time of 12 minutes with a strong absorption at 268 nm, which is typical for conjutrienes. The hydrolyzed lipid extracts of yeast cells which were transformed with the blank vector pYES2 and grown with 0.003% of linoleic acid show no fatty acids with a retention time of calendulic acid (not shown). Equally, the hydrolyzed lipid extracts of yeast cells which express the FAD2 gene contain no calendulic acid (Fig. 3B).

In contrast, the HPLC analysis of the extracts of pYES2-CalDes-transformed yeast cells grown with 0.003% of linoleic acid showed a signal with the retention time of calendulic acid (Fig. 3C), which also showed the same absorption spectrum as the standard with a maximum of 268 nm and secondary maxima of 258 and 282 nm (Fig. 4A, standard, and C, elution profile of *S. cerevisiae* INCSv1 transformed with Calendula officinalis pYES2-CalDes). It was thus demonstrated that the expression of calendulic acid desaturase in yeast results in the biosynthesis of calendulic acid. Calendulic acid from transformed yeast cells was only successfully detected after hydrolysis of the lipids. No calendulic acid was detected in the free fatty acids of these cells, that is to say that, in yeast, calendulic acid is incorporated into lipids. Since yeast contains no

triacylglycerides, it must be assumed that the detected calendulic acid had been bound in the phospholipids of the yeast.

In addition, the lipid extracts of transgenic yeast cells which simultaneously express FAD2 and CalDes also contain calendulic acid (not shown).

Example 6: Expression of calendulic acid desaturase in Arabidopsis thaliana and Linum usitatissimum

The expression of Calendula officinalis calendulic acid desaturase in transgenic plants is advantageous for increasing the calendulic acid content in these plants. To this end, the CalDes cDNA was cloned into binary vectors and transferred into A. thaliana and L. usitatissimum via Agrobacterium-mediated DNA transfer. The expression of the CalDes cDNA was under the control of the constitutive CaMV 35S promoter or the seed-specific USP promoter.

The expression vectors used were the vector pBinAR (Höfgen and Willmitzer, Plant Science, 66, 1990: 221 - 230) and the pBinAR derivative pBinAR-USP, in which the CaMV 35S promoter had been exchanged for the V. faba USP promoter. For recloning, the CalDes cDNA had to be excised from the vector pGEM-T. To this end, the latter was first cut with NcoI and filled up with Klenow to provide blunt ends; the insert was subsequently excised with SalI and cloned into the SmaI/SalI-cut vectors pBinAR and pBinAR-USP.

The resulting plasmids pBinAR-CalDes and pBinAR-USP-CalDes were transformed into Agrobacterium tumefaciens (Höfgen and Willmitzer, Nucl. Acids Res., 16, 1988: 9877). A. thaliana was transformed by "floral dip" (Clough and Bent, Plant Journal, 16, 1998: 735 - 743), and L. usitatissimum by coculturing linseed hypocotyl sections with transformed A. tumefaciens cells.

The expression of the CalDes gene in transgenic Arabidopsis and Linum plants was studied by Northern Blot analysis. Selected plants were studied for their calendulic acid content in the seed oil.

To achieve seed-specific expression of CalDes, it is also possible to use the napin promoter analogously to the USP promoter.

ART 34 AMDT 0050/50669

10/069772

JC19 Rec'd PCT/PTO 28 FEB 2002

We claim:

1. An isolated nucleic acid sequence which encodes a polypeptide with desaturase activity, selected from the following group:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1,

b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from the nucleic acid sequence shown in SEQ ID NO: 1,

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and which have at least 75% homology at amino acid level without substantially reducing the enzymatic activity of the polypeptides.

2. An amino acid sequence encoded by a nucleic acid sequence as claimed in claim 1.

3. An amino acid sequence as claimed in claim 2, encoded by the sequence shown in SEQ ID NO: 1.

4. A nucleic acid construct comprising a nucleic acid sequence as claimed in claim 1, where the nucleic acid sequence is linked to one or more regulatory signals.

5. A vector comprising a nucleic acid sequence as claimed in claim 1 or a nucleic acid construct as claimed in claim 4.

6. An organism comprising at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct as claimed in claim 4.

7. An organism as claimed in claim 6, which is a plant, a microorganism or an animal.

8. A transgenic plant comprising a functional or nonfunctional nucleic acid sequence as claimed in claim 1 or a functional or nonfunctional nucleic acid construct as claimed in claim 4.

9. A process for the preparation of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid

AMENDED SHEET

construct as claimed in claim 4 into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

10. A process for the preparation of triglycerides with an increased content of unsaturated fatty acids, which comprises introducing at least one nucleic acid sequence as claimed in claim 1 or at least one nucleic acid construct as claimed in claim 4 into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

11. A process for the preparation of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct as claimed in claim 4 into an oil-producing organism, growing this organism, isolating the oil contained in the organism and liberating the fatty acids contained in the oil.

12. A process for the preparation of triglycerides with an increased content of saturated fatty acids, which comprises introducing at least one nonfunctional nucleic acid sequence as claimed in claim 1 or at least one nonfunctional nucleic acid construct as claimed in claim 4 into an oil-producing organism, growing this organism and isolating the oil contained in the organism.

13. A process as claimed in claim 9 or 10, wherein the unsaturated fatty acids have an increased calendulic acid content.

14. A method as claimed in any of claims 9 to 12, wherein the organism is a plant or a microorganism.

15. An unsaturated fatty acid prepared by a process as claimed in claim 9.

16. A triglyceride with an increased content of unsaturated fatty acids prepared by a process as claimed in claim 10.

17. A saturated fatty acid prepared by a process as claimed in claim 11.

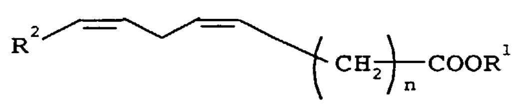
18. A triglyceride with an increased content of saturated fatty acids prepared by a process as claimed in claim 12.

19. The use of a nucleic acid sequence as claimed in claim 1 or of a nucleic acid construct as claimed in claim 4 for the generation of transgenic plants.

20. The use of a nucleic acid sequence as claimed in claim 1 or of a fragment thereof for isolating a genomic sequence via homology screening.

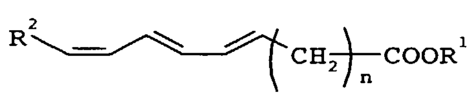
21. The use of unsaturated or saturated fatty acids as claimed in claim 15 or 17 or triglycerides with an increased content of unsaturated or saturated fatty acids as claimed in claim 16 or 18 for the preparation of foodstuffs, animal feed, cosmetics or pharmaceuticals.

22. An enzyme which is encoded by a nucleic acid sequence as claimed in claim 1 and which converts a fatty acid of the structure I,



(I)

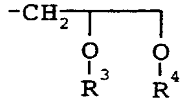
which has two double bonds separated from each other by a methylene group, to give a triunsaturated fatty acid of the structure II,



(II),

the three double bonds of the fatty acid being conjugated and the substituents and variables in the compounds of the structures I and II having the following meanings:

R^1 = hydrogen, substituted or unsubstituted, unsaturated or saturated, branched or unbranched C_1–C_{10}-alkyl-,



R^2 = substituted or unsubstituted, unsaturated or saturated C_1–C_9-Alkyl-

R^3 and R^4 independently of one another are hydrogen, substituted or unsubstituted, saturated or unsaturated, branched or unbranched C_1-C_{22}-alkylcarbonyl or phospho-,

n = 1 to 14

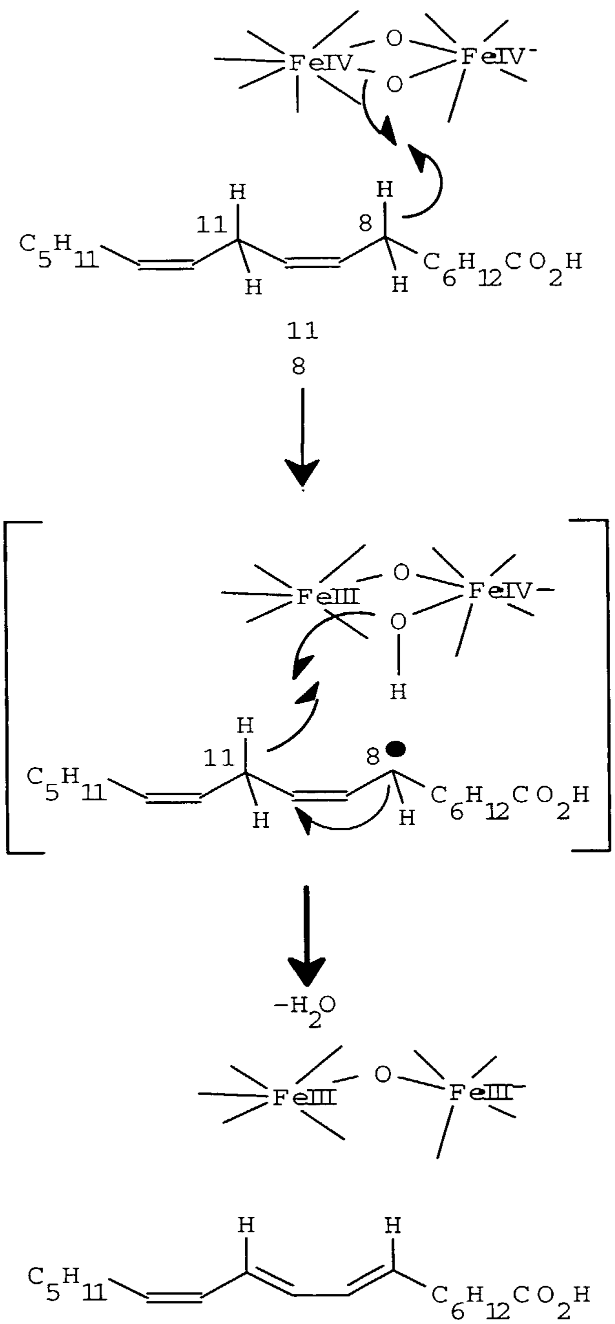
Fatty acid desaturase gene from plants

Abstract

The present invention relates to a process for the preparation of unsaturated or saturated fatty acids and a process for the preparation of triglycerides with an increased content of unsaturated or saturated fatty acids.

The invention furthermore relates to a nucleic acid sequence; a nucleic acid construct, a vector and organisms comprising at least one nucleic acid sequence or one nucleic acid construct. Furthermore, the invention relates to saturated or unsaturated fatty acids and triglycerides with an increased content of unsaturated or saturated fatty acids and their use.

Figur 1


FeIV
O
O
FeIV
H
H
11
8
C_5H_{11}
H
H
$C_6H_{12}CO_2H$
11
8
FeIII
O
FeIV
O
H
H
11
8
C_5H_{11}
H
H
$C_6H_{12}CO_2H$
$-H_2O$
FeIII
O
FeIII
H
H
C_5H_{11}
$C_6H_{12}CO_2H$

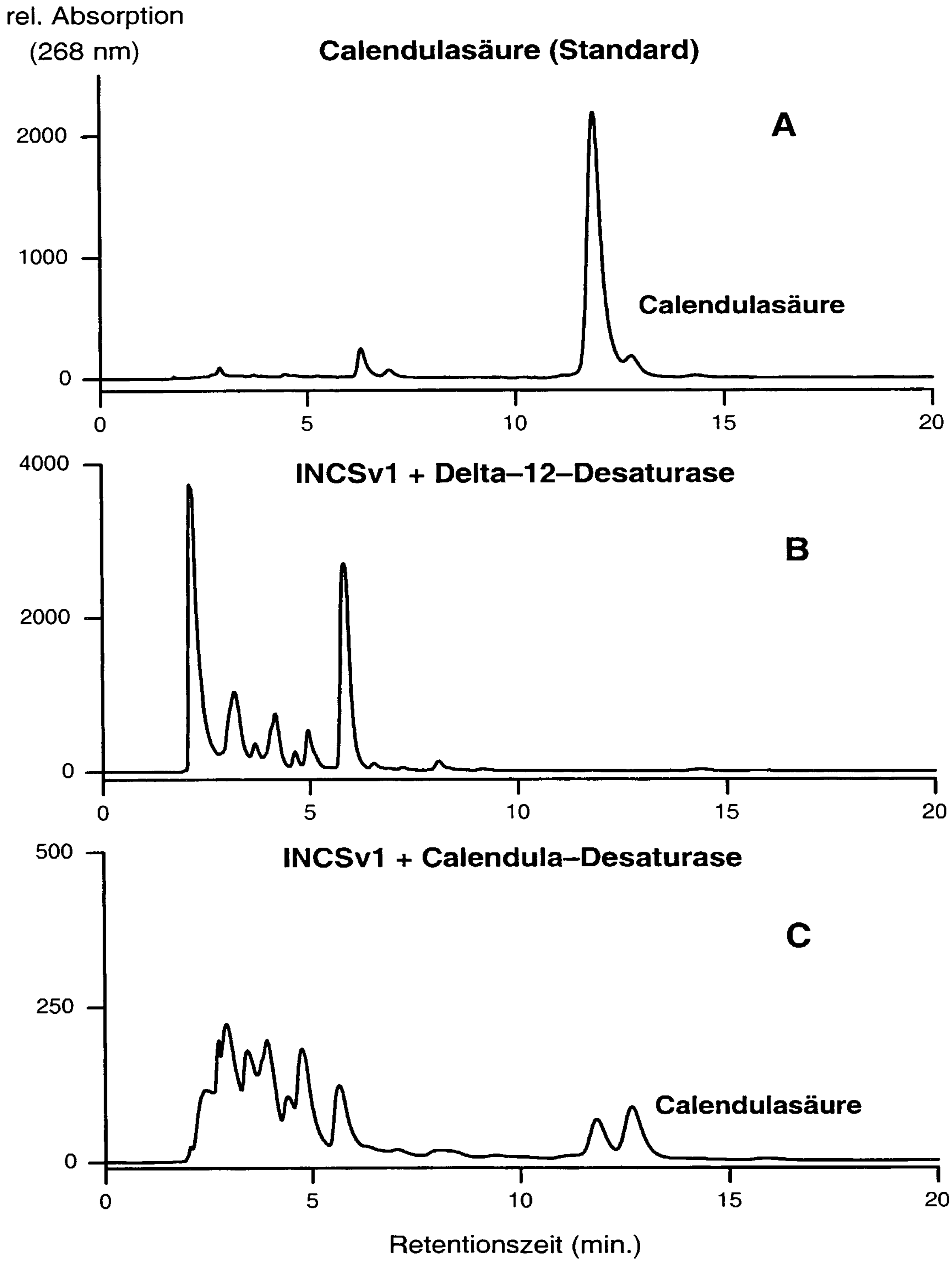
10/069772

Figur 2

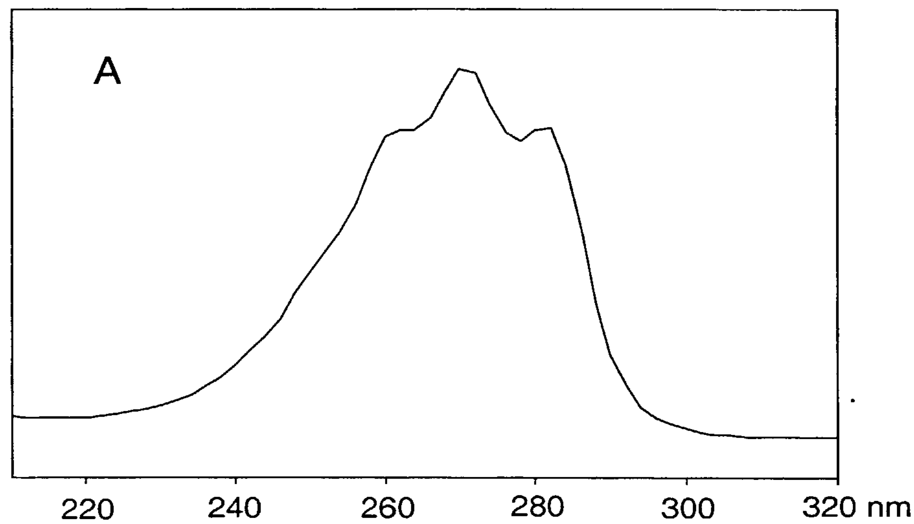
```
            1                                                  50
Co-CalDes   MGAGGRMSDP SEGK....NI LERVPVDP.P FTLSDLKKAI PTHCFERSVI
Ca-Acetyl   MGGGGR.GRT SQ.K....PL MERVSVDP.P FTVSDLKQAI PPHCFKRSVI
 Cp-Epoxy   MGAGGR.GRT SE.K....SV MERVSVDPVT FSLSELKQAI PPHCFQRSVI
   Bo-Des   MGGGGRMPVP TKGKKSKSDV FQRVPSEKPP FTVGDLKKVI PPHCFQRSVL

            51                                                100
Co-CalDes   RSSYYVVHDL IVAYVFYYLA NTYIPLIPTP LAYLAWPVYW FCQASILTGL
Ca-Acetyl   RSSYYIVHDA IIAYIFYFLA DKYIPILPAP LAYLAWPLYW FCQASILTGL
 Cp-Epoxy   RSSYYVVQDL IIAYIFYFLA NTYIPTLPTS LAYLAWPVYW FCQASVLTGL
   Bo-Des   HSFSYVVYDL VIAALFFYTA SRYIHLQPHP LSYVAWPLYW FCQGSVLTGV

            101                                               150
Co-CalDes   WVIGHECGHH AFSDYQLIDD IVGFVLHSAL LTPYFSWKYS HRNHHANTNS
Ca-Acetyl   WVIGHECGHH AFSDYQWVDD TVGFILHSFL MTPYFSWKYS HRNHHANTNS
 Cp-Epoxy   WILGHECGHH AFSNYTWFDD TVGFILHSFL LTPYFSWKFS HRNHHSNTSS
   Bo-Des   WVIAHECGHH AFSDYQWLDD TVGLLLHSAL LVPYFSWKYS HRRHHSNTGS

            151                                               200
Co-CalDes   LDNDEVYIPK RKSKVKIYSK LLNNPPGRVF TLVFRLTLGF PLYLLTNISG
Ca-Acetyl   LDNDEVYIPK SKAKVALYYK VLNHPPGRLL IMFITFTLGF PLYLFTNISG
 Cp-Epoxy   IDNDEVYIPK SKSKLARIYK LLNNPPGRLL VLIIMFTLGF PLYLLTNISG
   Bo-Des   LERDEVFVPK KRSGISWSSE YLNNPPGRVL VLLVQLTLGW PLYLMFNVSG

            201                                               250
Co-CalDes   KKYGRFANHF DPMSPIFNDR ERVQVLLSDF GLLAVFYAIK LLVAAKGAAW
Ca-Acetyl   KKYERFANHF DPMSPIFKER ERFQVLLSDL GLLAVLYGVK LAVAAKGAAW
 Cp-Epoxy   KKYDRFANHF DPMSPIFKER ERFQVFLSDL GLLAVFYGIK VAVANKGAAW
   Bo-Des   RPYDRFACHF DPKSPIYNDR ERLQIYISDA GIVAVMYGLY RLVAAKGVAW

            251                                               300
Co-CalDes   VINMYAIPVL GVSVFFVLIT YLHHTHLSLP HYDSTEWNWI KGALSTIDRD
Ca-Acetyl   VTCIYGIPVL GVFIFFDIIT YLHHTHLSLP HYDSSEWNWL RGALSTIDRD
 Cp-Epoxy   VACMYGVPVL GVFTFFDVIT FLHHTHQSSP HYDSTEWNWI RGALSAIDRD
   Bo-Des   VVCYYGVPLL VVNGFLVLIT YLQHTQPSLP HYDSSEWDWL KGALATVDRD

            301                                               350
Co-CalDes   FGFLNRVFHD VTHTHVLHHL ISYIPHYHAK EARDAIKPVL GEYYKIDRTP
Ca-Acetyl   FGFLNSVLHD VTHTHVMHHL FSYIPHYHAK EARDAINTVL GDFYKIDRTP
 Cp-Epoxy   FGFLNSVFHD VTHTHVMHHL FSYIPHYHAK EARDAIKPIL GDFYMIDRTP
   Bo-Des   YGFLNKVLHN ITDTHVAHHL FSTMPHYHAM EATKAIKPIL GDYYQCDRTP

            351                            384
Co-CalDes   IFKAMYREAK ECIYIEPDED SEHKGVFWY. HKM*
Ca-Acetyl   ILKAMWREAK ECIFIEPEKG RESKGVYWY. NKF*
 Cp-Epoxy   ILKAMWREGR ECMYIEPDS. .KLKGVYWY. HKL*
   Bo-Des   VFKAMYREVK ECIYVEADEG DNKKGVFWYK NKL*
```

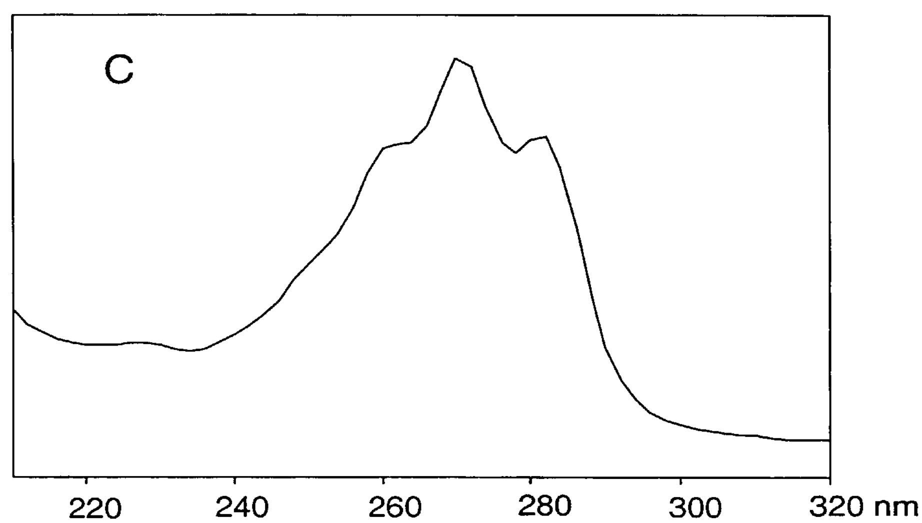
1064/99

Figur 3


rel. Absorption
(268 nm)
Calendulasäure (Standard)
A
2000
1000
0
Calendulasäure
0
5
10
15
20
4000
INCSv1 + Delta–12–Desaturase
B
2000
0
0
5
10
15
20
500
INCSv1 + Calendula–Desaturase
C
250
0
Calendulasäure
0
5
10
15
20
Retentionszeit (min.)

Figur 4





10/069772
JC19 Rec'd PCT/PTO 28 FEB 2002

SEQUENCE LISTING

```
<110> Department of plant biochemistry

<120> Fatty acid desaturase gene from plants

<130> Sequence_desaturase

<140> 50669
<141> 1999-08-31

<160> 2

<170> PatentIn Vers. 2.0

<210> 1
<211> 1285
<212> DNA
<213> Calendula officinalis

<220>
<221> CDS
<222> (42)..(1175)

<400> 1
aaaagctcac ttctctgtga gggtaattat atatcaacaa c atg ggt gct ggt ggt 56
                                              Met Gly Ala Gly Gly
                                                1               5

cgg atg tcg gat cca tct gag gga aaa aac atc ctt gaa cgt gtg cca   104
Arg Met Ser Asp Pro Ser Glu Gly Lys Asn Ile Leu Glu Arg Val Pro
                 10                  15                  20

gtc gat cca ccg ttc acg tta agc gat ctg aag aaa gcg att cct acc   152
Val Asp Pro Pro Phe Thr Leu Ser Asp Leu Lys Lys Ala Ile Pro Thr
             25                  30                  35

cat tgc ttt gag cga tct gtc atc cgg tca tca tac tat gtt gtt cat   200
His Cys Phe Glu Arg Ser Val Ile Arg Ser Ser Tyr Tyr Val Val His
         40                  45                  50

gat ctc att gtt gcc tat gtc ttc tac tac ctt gca aac acg tat atc   248
Asp Leu Ile Val Ala Tyr Val Phe Tyr Tyr Leu Ala Asn Thr Tyr Ile
     55                  60                  65

cct ctt att cct aca cct ctg gct tac cta gca tgg ccc gtt tac tgg   296
Pro Leu Ile Pro Thr Pro Leu Ala Tyr Leu Ala Trp Pro Val Tyr Trp
 70                  75                  80                  85

ttt tgt caa gct agc atc ctc acc ggc ctc tgg gtc atc ggt cac gaa   344
Phe Cys Gln Ala Ser Ile Leu Thr Gly Leu Trp Val Ile Gly His Glu
```

```
                    90                  95                  100

tgt ggt cac cat gca ttt agc gac tac cag ttg att gat gac att gtt    392
Cys Gly His His Ala Phe Ser Asp Tyr Gln Leu Ile Asp Asp Ile Val
            105                 110                 115

gga ttc gtg ctc cat tcg gct ctc ctc acc ccg tat ttc tct tgg aaa    440
Gly Phe Val Leu His Ser Ala Leu Leu Thr Pro Tyr Phe Ser Trp Lys
        120                 125                 130

tat agc cac agg aat cac cac gcc aac aca aat tca ctc gat aac gat    488
Tyr Ser His Arg Asn His His Ala Asn Thr Asn Ser Leu Asp Asn Asp
    135                 140                 145

gaa gtt tac att cct aaa cgt aag tcg aag gtc aag att tat tcc aaa    536
Glu Val Tyr Ile Pro Lys Arg Lys Ser Lys Val Lys Ile Tyr Ser Lys
150                 155                 160                 165

ctt ctt aac aat cca ccc ggg cga gtg ttc act ttg gtg ttt cgg ttg    584
Leu Leu Asn Asn Pro Pro Gly Arg Val Phe Thr Leu Val Phe Arg Leu
                170                 175                 180

act tta gga ttt ccg tta tac ctc tta act aat atc tcg ggc aag aaa    632
Thr Leu Gly Phe Pro Leu Tyr Leu Leu Thr Asn Ile Ser Gly Lys Lys
            185                 190                 195

tac ggg agg ttt gcc aac cac ttt gat ccc atg agt cca att ttc aac    680
Tyr Gly Arg Phe Ala Asn His Phe Asp Pro Met Ser Pro Ile Phe Asn
        200                 205                 210

gat cgt gaa cgc gtt caa gtt ttg cta tcc gat ttc ggt ctt ctc gct    728
Asp Arg Glu Arg Val Gln Val Leu Leu Ser Asp Phe Gly Leu Leu Ala
    215                 220                 225

gta ttt tat gca atc aag ctt ctt gta gca gca aaa ggg gca gct tgg    776
Val Phe Tyr Ala Ile Lys Leu Leu Val Ala Ala Lys Gly Ala Ala Trp
230                 235                 240                 245

gta atc aac atg tac gca att cca gta cta ggt gta agc gtg ttc ttc    824
Val Ile Asn Met Tyr Ala Ile Pro Val Leu Gly Val Ser Val Phe Phe
                250                 255                 260

gtt ttg atc aca tat ttg cac cac acc cat ctc tca ctc cct cat tat    872
Val Leu Ile Thr Tyr Leu His His Thr His Leu Ser Leu Pro His Tyr
            265                 270                 275

gat tca acc gaa tgg aac tgg atc aaa ggc gcc tta tca aca atc gat    920
Asp Ser Thr Glu Trp Asn Trp Ile Lys Gly Ala Leu Ser Thr Ile Asp
        280                 285                 290

agg gat ttc ggg ttc ctg aat cgg gtt ttc cac gac gtt aca cac act    968
```

Arg Asp Phe Gly Phe Leu Asn Arg Val Phe His Asp Val Thr His Thr
    295   300   305

cac gtc ttg cat cat ttg atc tca tac att cca cat tat cat gca aag   1016
His Val Leu His His Leu Ile Ser Tyr Ile Pro His Tyr His Ala Lys
310   315   320   325

gaa gca agg gat gca atc aag cca gtg ttg ggc gag tac tat aaa atc   1064
Glu Ala Arg Asp Ala Ile Lys Pro Val Leu Gly Glu Tyr Tyr Lys Ile
                330   335   340

gac agg act cca att ttc aaa gca atg tat aga gag gct aag gaa tgc   1112
Asp Arg Thr Pro Ile Phe Lys Ala Met Tyr Arg Glu Ala Lys Glu Cys
            345   350   355

atc tac atc gag ccc gat gag gat agc gag cac aaa ggt gtg ttc tgg   1160
Ile Tyr Ile Glu Pro Asp Glu Asp Ser Glu His Lys Gly Val Phe Trp
        360   365   370

tac cac aag atg taa tcaaaaaggt gtatgtcaat gcaattgtat gcttaattaa   1215
Tyr His Lys Met
    375

gttgttaaac tttctattcc gtgtaataaa ttatcattaa gagaaaaaaa aaaaaaaaaa 1275

aaaaaaaaaa   1285

<210> 2
<211> 377
<212> PRT
<213> Calendula officinalis

<400> 2
Met Gly Ala Gly Gly Arg Met Ser Asp Pro Ser Glu Gly Lys Asn Ile
  1   5   10   15

Leu Glu Arg Val Pro Val Asp Pro Pro Phe Thr Leu Ser Asp Leu Lys
             20   25   30

Lys Ala Ile Pro Thr His Cys Phe Glu Arg Ser Val Ile Arg Ser Ser
         35   40   45

Tyr Tyr Val Val His Asp Leu Ile Val Ala Tyr Val Phe Tyr Tyr Leu
     50   55   60

Ala Asn Thr Tyr Ile Pro Leu Ile Pro Thr Pro Leu Ala Tyr Leu Ala
 65   70   75   80

Trp Pro Val Tyr Trp Phe Cys Gln Ala Ser Ile Leu Thr Gly Leu Trp
                 85   90   95

Val Ile Gly His Glu Cys Gly His His Ala Phe Ser Asp Tyr Gln Leu
100 105 110

Ile Asp Asp Ile Val Gly Phe Val Leu His Ser Ala Leu Leu Thr Pro
115 120 125

Tyr Phe Ser Trp Lys Tyr Ser His Arg Asn His His Ala Asn Thr Asn
130 135 140

Ser Leu Asp Asn Asp Glu Val Tyr Ile Pro Lys Arg Lys Ser Lys Val
145 150 155 160

Lys Ile Tyr Ser Lys Leu Leu Asn Asn Pro Pro Gly Arg Val Phe Thr
165 170 175

Leu Val Phe Arg Leu Thr Leu Gly Phe Pro Leu Tyr Leu Leu Thr Asn
180 185 190

Ile Ser Gly Lys Lys Tyr Gly Arg Phe Ala Asn His Phe Asp Pro Met
195 200 205

Ser Pro Ile Phe Asn Asp Arg Glu Arg Val Gln Val Leu Leu Ser Asp
210 215 220

Phe Gly Leu Leu Ala Val Phe Tyr Ala Ile Lys Leu Leu Val Ala Ala
225 230 235 240

Lys Gly Ala Ala Trp Val Ile Asn Met Tyr Ala Ile Pro Val Leu Gly
245 250 255

Val Ser Val Phe Phe Val Leu Ile Thr Tyr Leu His His Thr His Leu
260 265 270

Ser Leu Pro His Tyr Asp Ser Thr Glu Trp Asn Trp Ile Lys Gly Ala
275 280 285

Leu Ser Thr Ile Asp Arg Asp Phe Gly Phe Leu Asn Arg Val Phe His
290 295 300

Asp Val Thr His Thr His Val Leu His His Leu Ile Ser Tyr Ile Pro
305 310 315 320

His Tyr His Ala Lys Glu Ala Arg Asp Ala Ile Lys Pro Val Leu Gly
325 330 335

Glu Tyr Tyr Lys Ile Asp Arg Thr Pro Ile Phe Lys Ala Met Tyr Arg
340 345 350

Glu Ala Lys Glu Cys Ile Tyr Ile Glu Pro Asp Glu Asp Ser Glu His
355 360 365

```
Lys Gly Val Phe Trp Tyr His Lys Met
    370                 375
```

SEQUENCE LISTING

```
<110> Feussner, Ivo
      Hornung, Ellen
      Fritsche, Kathrin
      Peitzsch, Nicola

<120> Fatty acid desaturase gene from plants

<130> 0050/50669

<140> US 09/721,756

<150> PCT/EP/00/08222
<151> 2000-08-23

<160> 2


<170> WordPerfect version 6.1


<210> 1
<211> 1285
<212> DNA
<213> Calendula officinalis

<220>
<221> CDS
<222> (42)..(1175)

<400> 1

aaaagctcac ttctctgtga gggtaattat atatcaacaa c atg ggt gct ggt ggt 56
                                              Met Gly Ala Gly Gly
                                               1               5

cgg atg tcg gat cca tct gag gga aaa aac atc ctt gaa cgt gtg cca   104
Arg Met Ser Asp Pro Ser Glu Gly Lys Asn Ile Leu Glu Arg Val Pro
                10                  15                  20

gtc gat cca ccg ttc acg tta agc gat ctg aag aaa gcg att cct acc   152
Val Asp Pro Pro Phe Thr Leu Ser Asp Leu Lys Lys Ala Ile Pro Thr
            25                  30                  35

cat tgc ttt gag cga tct gtc atc cgg tca tca tac tat gtt gtt cat   200
His Cys Phe Glu Arg Ser Val Ile Arg Ser Ser Tyr Tyr Val Val His
        40                  45                  50

gat ctc att gtt gcc tat gtc ttc tac tac ctt gca aac acg tat atc   248
Asp Leu Ile Val Ala Tyr Val Phe Tyr Tyr Leu Ala Asn Thr Tyr Ile
    55                  60                  65

cct ctt att cct aca cct ctg gct tac cta gca tgg ccc gtt tac tgg   296
Pro Leu Ile Pro Thr Pro Leu Ala Tyr Leu Ala Trp Pro Val Tyr Trp
70                  75                  80                  85

ttt tgt caa gct agc atc ctc acc ggc ctc tgg gtc atc ggt cac gaa   344
```

```
Phe Cys Gln Ala Ser Ile Leu Thr Gly Leu Trp Val Ile Gly His Glu
                90                  95                  100

tgt ggt cac cat gca ttt agc gac tac cag ttg att gat gac att gtt     392
Cys Gly His His Ala Phe Ser Asp Tyr Gln Leu Ile Asp Asp Ile Val
            105                 110                 115

gga ttc gtg ctc cat tcg gct ctc ctc acc ccg tat ttc tct tgg aaa     440
Gly Phe Val Leu His Ser Ala Leu Leu Thr Pro Tyr Phe Ser Trp Lys
        120                 125                 130

tat agc cac agg aat cac cac gcc aac aca aat tca ctc gat aac gat     488
Tyr Ser His Arg Asn His His Ala Asn Thr Asn Ser Leu Asp Asn Asp
    135                 140                 145

gaa gtt tac att cct aaa cgt aag tcg aag gtc aag att tat tcc aaa     536
Glu Val Tyr Ile Pro Lys Arg Lys Ser Lys Val Lys Ile Tyr Ser Lys
150                 155                 160                 165

ctt ctt aac aat cca ccc ggg cga gtg ttc act ttg gtg ttt cgg ttg     584
Leu Leu Asn Asn Pro Pro Gly Arg Val Phe Thr Leu Val Phe Arg Leu
                170                 175                 180

act tta gga ttt ccg tta tac ctc tta act aat atc tcg ggc aag aaa     632
Thr Leu Gly Phe Pro Leu Tyr Leu Leu Thr Asn Ile Ser Gly Lys Lys
            185                 190                 195

tac ggg agg ttt gcc aac cac ttt gat ccc atg agt cca att ttc aac     680
Tyr Gly Arg Phe Ala Asn His Phe Asp Pro Met Ser Pro Ile Phe Asn
        200                 205                 210

gat cgt gaa cgc gtt caa gtt ttg cta tcc gat ttc ggt ctt ctc gct     728
Asp Arg Glu Arg Val Gln Val Leu Leu Ser Asp Phe Gly Leu Leu Ala
    215                 220                 225

gta ttt tat gca atc aag ctt ctt gta gca gca aaa ggg gca gct tgg     776
Val Phe Tyr Ala Ile Lys Leu Leu Val Ala Ala Lys Gly Ala Ala Trp
230                 235                 240                 245

gta atc aac atg tac gca att cca gta cta ggt gta agc gtg ttc ttc     824
Val Ile Asn Met Tyr Ala Ile Pro Val Leu Gly Val Ser Val Phe Phe
                250                 255                 260

gtt ttg atc aca tat ttg cac cac acc cat ctc tca ctc cct cat tat     872
Val Leu Ile Thr Tyr Leu His His Thr His Leu Ser Leu Pro His Tyr
            265                 270                 275

gat tca acc gaa tgg aac tgg atc aaa ggc gcc tta tca aca atc gat     920
Asp Ser Thr Glu Trp Asn Trp Ile Lys Gly Ala Leu Ser Thr Ile Asp
        280                 285                 290

agg gat ttc ggg ttc ctg aat cgg gtt ttc cac gac gtt aca cac act     968
Arg Asp Phe Gly Phe Leu Asn Arg Val Phe His Asp Val Thr His Thr
    295                 300                 305

cac gtc ttg cat cat ttg atc tca tac att cca cat tat cat gca aag    1016
His Val Leu His His Leu Ile Ser Tyr Ile Pro His Tyr His Ala Lys
310                 315                 320                 325
```

gaa gca agg gat gca atc aag cca gtg ttg ggc gag tac tat aaa atc 1064
Glu Ala Arg Asp Ala Ile Lys Pro Val Leu Gly Glu Tyr Tyr Lys Ile
330 335 340

gac agg act cca att ttc aaa gca atg tat aga gag gct aag gaa tgc 1112
Asp Arg Thr Pro Ile Phe Lys Ala Met Tyr Arg Glu Ala Lys Glu Cys
345 350 355

atc tac atc gag ccc gat gag gat agc gag cac aaa ggt gtg ttc tgg 1160
Ile Tyr Ile Glu Pro Asp Glu Asp Ser Glu His Lys Gly Val Phe Trp
360 365 370

tac cac aag atg taa tcaaaaaggt gtatgtcaat gcaattgtat gcttaattaa 1215
Tyr His Lys Met
375

gttgttaaac tttctattcc gtgtaataaa ttatcattaa gagaaaaaaa aaaaaaaaaa 1275

aaaaaaaaaa 1285

<210> 2
<211> 377
<212> PRT
<213> Calendula officinalis

<400> 2

Met Gly Ala Gly Gly Arg Met Ser Asp Pro Ser Glu Gly Lys Asn Ile
1 5 10 15

Leu Glu Arg Val Pro Val Asp Pro Pro Phe Thr Leu Ser Asp Leu Lys
20 25 30

Lys Ala Ile Pro Thr His Cys Phe Glu Arg Ser Val Ile Arg Ser Ser
35 40 45

Tyr Tyr Val Val His Asp Leu Ile Val Ala Tyr Val Phe Tyr Tyr Leu
50 55 60

Ala Asn Thr Tyr Ile Pro Leu Ile Pro Thr Pro Leu Ala Tyr Leu Ala
65 70 75 80

Trp Pro Val Tyr Trp Phe Cys Gln Ala Ser Ile Leu Thr Gly Leu Trp
85 90 95

Val Ile Gly His Glu Cys Gly His His Ala Phe Ser Asp Tyr Gln Leu
100 105 110

Ile Asp Asp Ile Val Gly Phe Val Leu His Ser Ala Leu Leu Thr Pro
115 120 125

Tyr Phe Ser Trp Lys Tyr Ser His Arg Asn His His Ala Asn Thr Asn
130 135 140

Ser Leu Asp Asn Asp Glu Val Tyr Ile Pro Lys Arg Lys Ser Lys Val
145 150 155 160

```
Lys Ile Tyr Ser Lys Leu Leu Asn Asn Pro Pro Gly Arg Val Phe Thr
                165                 170                 175

Leu Val Phe Arg Leu Thr Leu Gly Phe Pro Leu Tyr Leu Leu Thr Asn
            180                 185                 190

Ile Ser Gly Lys Lys Tyr Gly Arg Phe Ala Asn His Phe Asp Pro Met
        195                 200                 205

Ser Pro Ile Phe Asn Asp Arg Glu Arg Val Gln Val Leu Leu Ser Asp
    210                 215                 220

Phe Gly Leu Leu Ala Val Phe Tyr Ala Ile Lys Leu Leu Val Ala Ala
225                 230                 235                 240

Lys Gly Ala Ala Trp Val Ile Asn Met Tyr Ala Ile Pro Val Leu Gly
                245                 250                 255

Val Ser Val Phe Phe Val Leu Ile Thr Tyr Leu His His Thr His Leu
            260                 265                 270

Ser Leu Pro His Tyr Asp Ser Thr Glu Trp Asn Trp Ile Lys Gly Ala
        275                 280                 285

Leu Ser Thr Ile Asp Arg Asp Phe Gly Phe Leu Asn Arg Val Phe His
    290                 295                 300

Asp Val Thr His Thr His Val Leu His His Leu Ile Ser Tyr Ile Pro
305                 310                 315                 320

His Tyr His Ala Lys Glu Ala Arg Asp Ala Ile Lys Pro Val Leu Gly
                325                 330                 335

Glu Tyr Tyr Lys Ile Asp Arg Thr Pro Ile Phe Lys Ala Met Tyr Arg
            340                 345                 350

Glu Ala Lys Glu Cys Ile Tyr Ile Glu Pro Asp Glu Asp Ser Glu His
        355                 360                 365

Lys Gly Val Phe Trp Tyr His Lys Met
    370                 375
```

Declaration, Power of Attorney and Petition

0050/050669

Customer No.

26474

PATENT TRADEMARK OFFICE

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

FATTY ACID DESATURASE GENE FROM PLANTS

the specification of which

[ ] is attached hereto.

[ ] was filed on ______________________________ as

Application Serial No. ______________________________

and amended on ______________________________ .

[x] was filed as PCT international application

Number *PCT/EP/00/08222*______________________________

on *23 August 2000*______________________________ ,

and was amended under PCT Article 19

on ______________________________ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
19941609.5	*Germany*	*01 September 1999*	[x] Yes [ ] No

pdusksp001 - 25

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)

And we (I) hereby appoint **Messrs. HERBERT. B. KEIL**, Registration Number 18,967; and **RUSSEL E. WEINKAUF**, Registration Number 18, 495; the address of both being Messrs. Keil & Weinkauf, 1101 Connecticut Ave., N.W., Washington, D.C. 20036 (telephone 202–659–0100), our attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to sign the drawings, to receive the patent, and to transact all business in the Patent Office connected therewith.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

pduskpct001 - 25

1-10 *Ivo Feussner*
NAME OF INVENTOR

Signature of Inventor

Date September 7, 2000

Schleiermacherstr. 9
06114 Halle
Germany DEX
Citizen of: Germany
Post Office Address: same as residence

2-10 *Ellen Hornung*
NAME OF INVENTOR

Signature of Inventor

Date September 7, 2000

Thomasiusstr. 44
06110 Halle
Germany DEX
Citizen of: Germany
Post Office Address: same as residence

3-10 *Kathrin Fritsche*
NAME OF INVENTOR

Signature of Inventor

Date September 7, 2000

Rodenburgstraat 65
6811 HN Arnhem
Netherlands NLX
Citizen of: Germany
Post Office Address: same as residence

4-10 *Nicola Peitzsch*
NAME OF INVENTOR

Signature of Inventor

Date September 7, 2000

W.–Seelenbinder–Str. 34
39118 Magdeburg
Germany DEX
Citizen of: Germany
Post Office Address: same as residence

---

pduskpct001 - 25

5-D *Andreas Renz*
NAME OF INVENTOR

Signature of Inventor

Date September 7, 2000

Heinrich–von–Kleist–Str.6
67117 Limburgerhof
Germany DEX
Citizen of: Germany
Post Office Address: same as residence

pduskpct001 - 25